

FTIR spectroscopy of bacteriorhodopsin microcrystals

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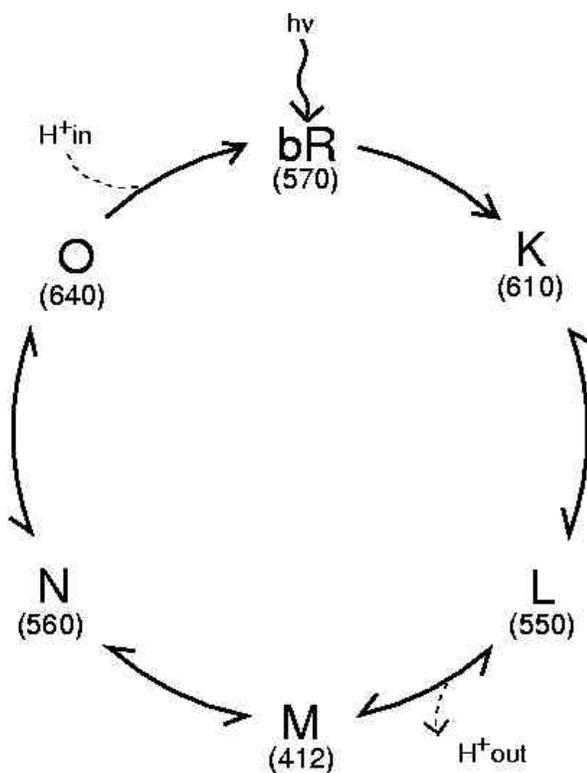
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INTRODUCTION

Bacteriorhodopsin (bR) is the sole protein component of the purple membrane of *Halobacterium salinarium* [1]. The function of bR *in vivo* is to convert solar energy into a pH gradient across the cell membrane which the organism uses to drive ATP synthesis [2]. Bacteriorhodopsin undergoes a light-induced cycle of physicochemical changes for every proton it pumps out of the cell. The photocycle (Figure 1) of bR has been well-characterized by both visible and IR spectroscopy. The major intermediates are identified as the K, L, M, N and O intermediates, and each has a distinct visible color and a distinct IR spectrum [3]. The Schiff base that connects the side chain of Lys 216 to the retinal molecule buried within the core of the apoprotein [4] is deprotonated upon formation of the M intermediate, and reprotonated when the M intermediate decays. Since access to the Schiff base is believed to switch from the extracellular side of the membrane to the cytoplasmic side between these two proton transfer events, the M intermediate is of particular interest.



High resolution x-ray diffraction experiments on microcrystals of bR have recently become possible, through the discovery by Landau and Rosenbusch that the solubilized protein can be crystallized from the bicontinuous lipid-water gel that is formed by mono-olein [5]. Structural studies on intermediate states of the photocycle thus become a high priority, allowing the visualization of the structural changes that are responsible for converting light energy into a proton-motive force. We have collected extensive diffraction data on bR crystals trapped in the M state, and refinement of the atomic model of this intermediate is nearly complete; data collection from crystals trapped in the L state is underway. We have also collected diffraction data from the resting state of crystals of the F219L mutant, and we intend to use those crystals to trap the N intermediate in the near future. It is crucial, however, that we use IR spectroscopy on bR microcrystals to confirm that we do trap the desired photointermediate in the protein microcrystals, and to define the experimental conditions under which is possible to trap them.

Figure 1. Key steps in the photocycle of bacteriorhodopsin. Photoexcitation of the light-adapted resting state (bR570) initiates the photocycle, represented by the five major photointermediates. The optical absorbance maximum of each is given in parentheses; photon exchange with the bulk aqueous phases on either side of the membrane is also indicated.

RESULTS

Early experiments led us to design a sealed, vacuum-tight sample holder that allows us to study hydrated crystals at low temperatures. Using this sample holder, we have achieved our first goal of trapping the M-state intermediate by illuminating bacteriorhodopsin crystals with yellow light at 230K (Figure 2). Characteristic IR difference bands (see annotations) confirm that an early phase of the M intermediate was trapped under conditions as near as possible to those used in our x-ray diffraction experiments. Our next objective is to confirm the trapping of the L state at 170K; preliminary results suggest that we have indeed succeeded in doing so.

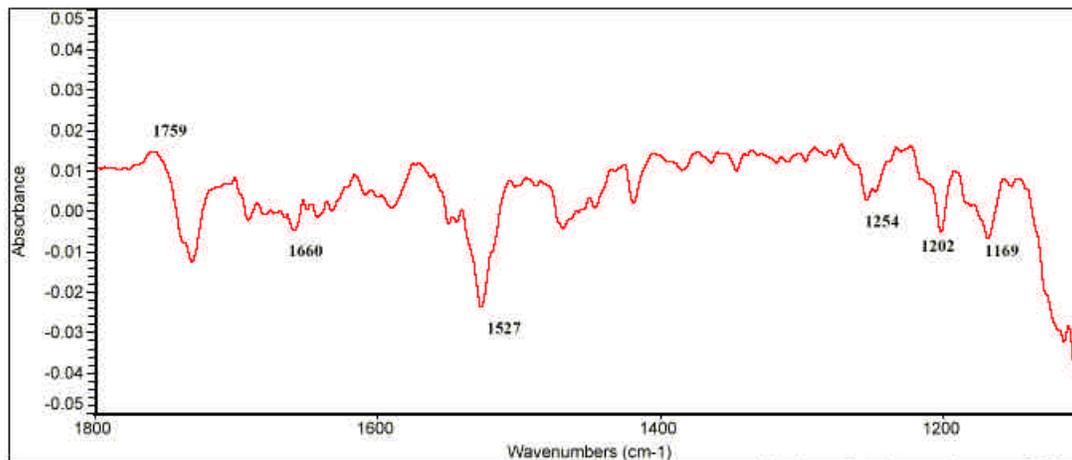


Figure 2. M-br570 FTIR difference spectrum. IR spectra at a resolution of 4 cm^{-1} were collected at Beamline 1.4.3 from bacteriorhodopsin specimens cooled to 230K and either illuminated with yellow light to form the M intermediate, or not, and arithmetically averaged. The two data sets were then subtracted. Annotations identify salient features of the spectrum: 1) the positive band at $\sim 1761\text{ cm}^{-1}$ signifies the protonation of Asp 85; 2) the negative band at $\sim 1660\text{ cm}^{-1}$ in the amide I region is an indicator of the early phase of the M intermediate; 3) the negative bands at $\sim 1528\text{ cm}^{-1}$ and between 1260 and 1160 cm^{-1} reflect the isomerization of the retinal group; and 4) the absence of a positive peak at 1183 cm^{-1} denotes the deprotonation of the Schiff base. Features 1) and 4) are diagnostic for the M intermediate.

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